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Factors affecting *in vitro* propagation of some genotypes of Himalayan cedar [*Cedrus deodara* (Roxb. ex Lamb) G. Don.]

Z. Nazemi Rafi, H. Salehi (*)
Department of Horticultural Science, School of Agriculture, Shiraz University, Shiraz, Iran.

Key words: auxin, conifers, cytokinins, micropropagation, pinaceae.

Abstract: Four genotypes of Himalayan cedar were grown *in vitro* for assessing shoot proliferation. This experiment consist three parts. Initially, the explants (leafy and defoliated shoot-tips) of mature plants were disinfected and cultured on different basal media (LP, MS and WPM) that supplemented with benzyladenine (BA) at different concentrations for 6 weeks. Leafless explants produced the highest number of shoots and the longest shoots for four genotypes. There was no significant difference between the culture mediums and benzyladenine concentrations. In second phase, the influence of benzyladenine (2.5, 5, 10, 20 µM) and thidiazuron (TDZ) (0.4, 0.8, 1.6 µM) with combination of different auxin (NAA) concentrations (0, 1, 2, 3 µM) was determined on axillary shoot proliferation of the leafless explants of four genotypes grown on WPM. For all thidiazuron concentrations, significant differences between genotypes were detected. In general, with all genotypes, the use of 0.8 µM thidiazuron in the absence or presence of auxin (2 µM) led to the highest length and number of axillary shoots per explant, respectively. Finally, in another experiment, the following cytokinin treatments were investigated for axillary shoot multiplication of the CD1 genotype: thidiazuron (0, 0.1, 0.2, 0.4, 0.8 µM) and N6-[2-Isopentenyl] adenine (2iP) (0, 0.1, 0.2, 0.3, 0.4, 0.5 µM) in combination with benzyladenine (2.5 µM). The best results were obtained in thidiazuron (0.8 µM) with combination of benzyladenine (2.5 µM). This protocol is considered as the first successful report on culture establishment of some genotypes of mature *C. deodara* trees.

(*) **Corresponding author:**
hsalehi@shirazu.ac.ir

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

The Himalayan cedar [*Cedrus deodara* (Roxb. ex Lamb) G. Don.], which belongs to the Pinaceae family, is a beautiful, evergreen, and ornamental tree growing widely on the gradient of the Western Himalayas (Champion *et al.*, 1965). Commercial seed bearing of *C. deodara* begins about 30 to 45 years of age, and good seed crops are borne every 3 years (Tewari, 1994). Regeneration through seeds in *Cedrus deodara* is quite slow and undependable. Generally, there is a preference for propagation of mature trees, which helps improve afforestation management, breeding projects and production of elite tree genotypes. However, maturation of most tree species is a major limiting factor for the use of micropropagation in

afforestation projects (Lin *et al.*, 1991). There are a few reports on *in vitro* propagation of mature conifers in the last 20 years (Gupta and Durzan, 1985; Dumas and Monteuuis, 1995; Parasharami *et al.*, 2003; Andersone and Ievinsh, 2005; Malabadi and van Staden, 2005; Cortizo *et al.*, 2009; De Diego *et al.*, 2010). Few reports on *in vitro* propagation of cedars (*Cedrus libani* A. Rich. and *C. atlantica* Manetti) are available (Piola and Rohr, 1996; Piola *et al.*, 1998, 1999; Renau-Morata *et al.*, 2005). There are only two reports on the *in vitro* culture of deodar cedar with the sole use of the seeds (Bhatnagar *et al.*, 1983; Tamta and Palni, 2004).

Among regeneration methods, axillary bud induction is preferable for most maintenance of genetic stability and less mutation risk (Vasil and Vasil, 1980; Pierik, 1987). The enhancement of reliability of tissue culture system is being achieved through improving of medium component or correction of the conditions of environment, or both. However, the genotype has a significant impact on the accuracy and repeatability of tissue culture and its effect must be evaluated (Sul and Korban, 1994). In this domain, cytokinins and auxins play important roles for maintenance and acceptable growth of cultures. During the recent decades, several synthetic compounds have been introduced for induction of regeneration potential of plants (Guo *et al.*, 2011). Among the compounds, TDZ (Tang and Newton, 2005) and BA (Datta *et al*., 2006) are highly effective on Pinaceae family such as Eastern white pine and lodgepole pine, Virginia pine, red spruce, and *Taxus wallichiana* Zucc., respectively.

To our best knowledge, no report document the *in vitro* vegetative regeneration of this species from mature tissues. Our goal was to build methods for the *in vitro* propagation of *C. deodara* from adult trees, and to show the effect of genotype on its micropropagation.

2. Materials and Methods

Experiment 1. Plant materials

Actively growing shoots (4-6 cm long) were collected from mature (20-25 years old) *C. deodara* trees (genotype not specified) in a seed orchard near the School of Agriculture, Shiraz University, Iran. This was done from September 2015 to November 2016. The shoots were wrapped with wet paper toweling, enclosed in plastic bags and then kept at 4°C until 1 day before use.

Surface sterilization of explants

In this experiment, two kinds of explants with the similar length were used. The first type of explants retained their needles (leaves) to full length (Fig. 1 a). In the second type of explants, the leaves were trimmed to a quarter of their initial length (Fig. 1 a). Both types of explants were soaked in tap-water for at least 2 h. Then, they were submerged for 30 minutes in an aqueous solution of 2% benomyl to reduce fungal contamination. Afterwards, they were treated with 70% ethanol for 2 minutes followed by 15% Clorox (containing 5.25% sodium hypochlorite) with 0.2% 'Tween-20' for 15 minutes for surface sterilization. Finally, they were rinsed three times with sterile distilled water. Both kinds of explants were cut into 1-2 cm pieces under sterile conditions and subsequently cultured on different nutrient media.

Culture establishment

The explants were cultured on three different culture media: LP (Quoirin and Lepoivre, 1977), woody plant medium [WPM] (Lloyd and McCown, 1980) and MS (Murashige and Skoog, 1962), that were supplemented with benzyladenine (BA) at different concentrations (0, 0.63, 1.25, 2.5, 5 µM). Then, the cultures were placed in a growth chamber at 25±2°C under a 16 h photoperiod provided by cool white fluorescent lamps (30 µm m^{-2} s^{-1}) for 6 weeks. At the end, the number and length of proliferated shoots were measured.

Experiment 2. Plant materials and aseptic culture

This experiment consisted of two phases. In the first phase, the effect of different growth regulators on axillary shoot proliferation of four genotypes was

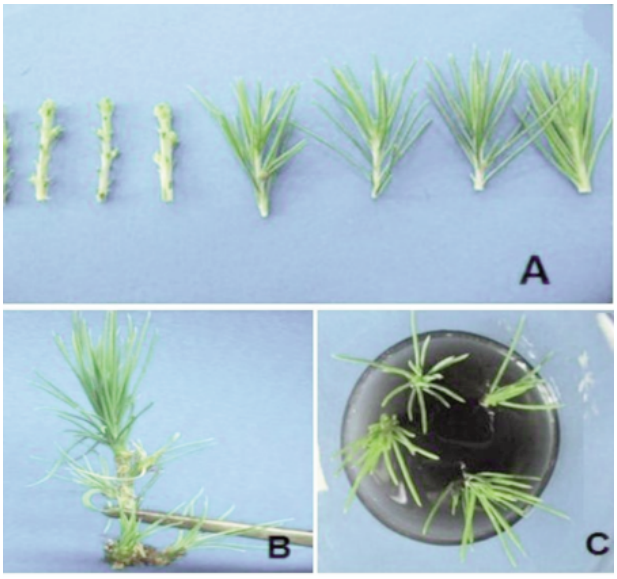

Fig. 1 - *In vitro* proliferation of *Cedrus deodara* (Roxb. ex Lamb) G. Don. (A) Different types of explants: explants retained their needles (leaves) and, defoliated explants; (B) Axillary shoot proliferation on WPM medium containing 0.8 µM TDZ and 2 µM NAA (after 4 weeks); (C) Elongation of axillary bud on EM medium after 90 days.

studied. In the second phase, axillary shoot multiplication of genotype CD1 was studied. To examine the effect of genotype on proliferation rate, 110 to 130 actively growing shoots with 4-6 cm length were collected from four mature 20-25 year-old *C. deodara* trees (genotypes CD1 to CD4) in a seed orchard near the School of Agriculture, Shiraz University, Iran. The shoots were defoliated, cut into 1-2 cm in length pieces and used as initial explants (n=114 per genotype). Surface sterilization was carried out in the same manner as in experiment 1. For culture establishment, the explants were cultured on WPM medium supplemented with growth regulators [BA, NAA and thidiazuron (TDZ)] for shoot induction and proliferation. The medium was supplemented with BA (0, 2.5, 5, 10, 20 µM) and TDZ (0, 0.4, 0.8, 1.6 µM) alone or in combination with NAA (1, 2, 3 µM). The effect of TDZ and BA on shoot proliferation of four genotypes (CD1 to CD4) was investigated. The conditions, under which the cultures were incubated, were same to those of experiment 1.

Elongation of induced shoots

Axillary shoots formed on explants (from genotype CD1) were isolated and transferred to elongation media (EM). The EM was growth regulator-free half strength WPM, and it was supplemented with 3 g L^{-1} activated charcoal (AC), 15 g L^{-1} sucrose and 8 g L^{-1} agar. The explants were maintained in the culture medium, and then subcultured into glass jars (150 ml) containing 40 ml of the fresh EM. The environmental conditions were same as that in culture establishment. The subculturing procedure was repeated every 4 weeks for 3 months. The culture conditions (temperature and light) were same as those used for culture establishment experiment.

Shoot multiplication

After 3 month, the shoots grown on EM were cut to the same length and then transferred to shoot multiplication medium containing TDZ (0, 0.1, 0.2, 0.4, 0.8 µM) and 2iP (0, 0.1, 0.2, 0.3, 0.4, 0.5 µM) in combination with 2.5 µM BA for 6 weeks.

Statistical analysis

All experiments were conducted as factorial based on a completely randomized design with four replications, each replicate comprised of four jam glasses, four explants per glass. All experiments were repeated twice. Shoot proliferation rate and shoot length were recorded at the end of the fourth week. SPSS statistical software was used for analyzing data, and the one-way ANOVA with Tukey's test (*P<0.05*) was used for comparing means. Three-way ANOVA was applied to examine the interactions of TDZ, BA and genotype.

3. Results

Experiment 1

The effects of different culture media (LP, MS and WPM) on two types of explants were investigated. The defoliated explants showed the best results, and there was a significant difference for shoot proliferation between defoliated and leafy explants, in all three culture media. The lowest frequency in shoot proliferation belonged to the leafy explants in MS medium (0.06). According to the results, WPM medium showed the highest number and length of proliferated shoots, but without significant difference as compared with LP. Moreover, leafless explants produced higher and longer proliferated shoots than leafy explants. There was no significant difference between different amounts of BA on length and number of proliferated shoots (data not shown).

Experiment 2

When all four genotypes (CD1, CD2, CD3, CD4) were grown at different levels of cytokinin treatments, shoot proliferation from leafless explants was observed. The highest mean number of shoots per explant was observed at 0.8 µM thidiazuron for genotypes CD1 (4.6), CD2 (4.4), CD3 (2.4), and at 0.4 µM thidiazuron for CD4 (2.4) (Fig. 2). Genotypes CD1 and CD2 were the most responsive genotypes over all cytokinin treatments. Furthermore, the highest

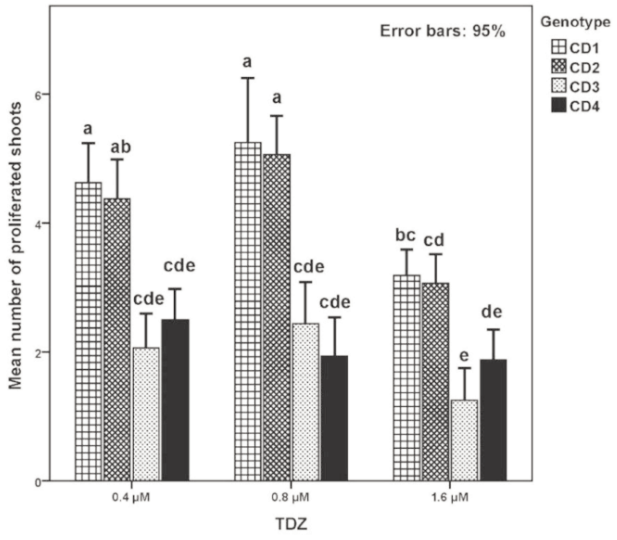

Fig. 2 - Effect of TDZ concentrations on shoot proliferation of four genotypes of *Cedrus deodara*. Bars with the same letters indicate no significant difference at Tukey test (P=0.05).

mean number of axillary shoots per explant of all genotypes (4.31) was obtained when 0.8 µM TDZ was used in combination with 2 µM NAA and the highest mean length of axillary shoots of all genotypes (11.2 mm) was obtained when 0.8 µM TDZ was applied alone (Tables 1, 2, Fig. 1 b). NAA had a positive effect on quality attributes (like color, vitality, and size). However, increasing the concentration of NAA to 3 µM reduced the number and length of proliferated shoots (Tables 1, 2). Then, axillary shoot multiplication of genotype CD1 was studied (Fig. 3). The highest mean number of axillary shoots per explant (4.13) and mean length of axillary shoots (10.38 mm) were obtained when 0.8 µM TDZ was applied (Table 3).

The best result of shoot proliferation obtained for CD1 genotype on WPM medium supplemented with 0.8 µM TDZ (Fig. 2). The same result was obtained for CD1 genotype on WPM medium supplemented with 2.5 µM BA (Fig. 4).

4. Discussion and Conclusions

Propagation of conifers from mature explants has always been difficult. In general, most studies on the induction of embryogenesis and/or organogenesis in conifers includes the culture of seed or zygotic tis-

Table 1 - Effects of different concentrations of TDZ, BA and NAA on the number of proliferated shoots in *Cedrus deodara*

Treatments	NAA				Mean values
	0	1	2	3	
Control	2.41± 0.02 lm†	2.16±0.02 pq	2.31±0.05 mno	1.97±0.01 r	2.21 F
TDZ (µM)					
0.4	3.75±0.00 b	3.31±0.03 e	3.44±0.03 de	3.06±0.03 f	3.39 B
0.8	3.38±0.03 de	3.50±0.00 cd	4.31±0.03 a	3.63±0.03 bc	3.70 A
1.6	2.25±0.00 nop	2.50±0.00 jkl	2.63±0.03 ij	2.00±0.00 r	2.34 EF
BA (µM)					
2.5	2.69±0.03 hi	3.06±0.03 f	3.40±0.03 de	2.50±0.00 jkl	2.91 C
5	2.38±0.03 lmn	2.44±0.03 klm	2.44±0.03 klm	2.56±0.03 ijk	2.45 E
10	2.44±0.03 klm	2.88±0.03 g	2.81±0.03 gh	2.31±0.03 mno	2.61 D
20	2.25±0.00 nop	2.38±0.03 lmn	2.19±0.03 opq	2.06±0.03 qr	2.22 F
Mean values	2.66 B	2.71 AB	2.87 A	2.45 C	2.67

Means with the same letters (small letters for interactions and capital letters for main effects) indicate no significant difference at Tukey's test (P=0.05).

Table 2 - Effects of different concentrations of TDZ, BA and NAA on the length of proliferated shoots in *Cedrus deodara*

Treatments	NAA				Mean values
	0	1	2	3	
Control					
0.0	5.80±0.06 h	5.55±0.06 hij	4.88±0.04 klm	4.50±0.09 mn	5.18 D
TDZ (µM)					
0.4	8.75±0.05 c	6.92±0.01 fg	8.12±0.09 d	7.12±0.12 fg	7.73 B
0.8	11.20±0.14 a	7.75±0.07 de	9.56±0.05 b	6.81±0.22 g	8.83 A
1.6	4.96±0.07 klm	5.24±0.08 ijk	5.20±0.08 i-l	4.67±0.02 lm	5.02 D
BA (µM)					
2.5	6.82±0.16 g	7.32±0.10 efg	5.57±0.10 hij	5.08±0.08 jkl	6.20 C
5	7.15±0.19 fg	7.43±0.10 ef	5.93±0.11 h	5.18±0.15 i-l	6.42 C
10	5.65±0.16 hi	5.93±0.08 h	4.07±0.09 no	3.12±0.08 q	4.69 D
20	3.44±0.12 pq	3.84±0.17 op	3.90±0.13 op	2.16±0.07 r	3.34 E
Mean values	6.62 A	6.17 AB	5.79 B	4.79 C	5.84

Means with the same letters (small letters for interactions and capital letters for main effects) indicate no significant difference at Tukey's test (P=0.05).

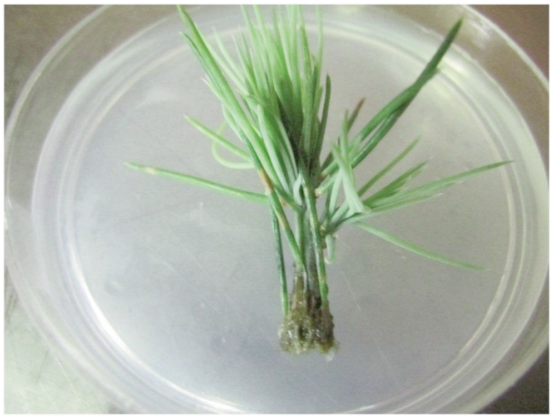

Fig. 3 - Multiple shoot formation on subculture of *Cedrus deodara* on WPM with 0.8 µM TDZ in combination with 2.5 µM BA.

Table 3 - Effects of different concentrations of TDZ and 2iP in combination with 2.5 µM BA on shoot multiplication of *Cedrus deodara*

Treatments	Number of proliferated shoots	Length
Control		
0.0	2.44± 0.07 e	6.69± 0.12 e
TDZ (µM)		
0.1	3.38± 0.16 bc	7.26± 0.16 de
0.2	3.44± 0.21 bc	7.80± 0.27 cd
0.4	3.88± 0.16 ab	9.93± 0.24 a
0.8	4.13± 0.13 a	10.38± 0.11 a
2iP (µM)		
0.1	2.50± 0.20 e	6.58± 0.11 e
0.2	2.69± 0.12 de	7.38± 0.18 de
0.3	3.00± 0.10 cde	7.93± 0.21 cd
0.4	3.25± 0.18 bcd	8.38± 0.15 bc
0.5	3.56± 0.12 abc	8.88± 0.14 b

In each column, means with the same letters indicate no significant difference at Tukey's test (P=0.05).

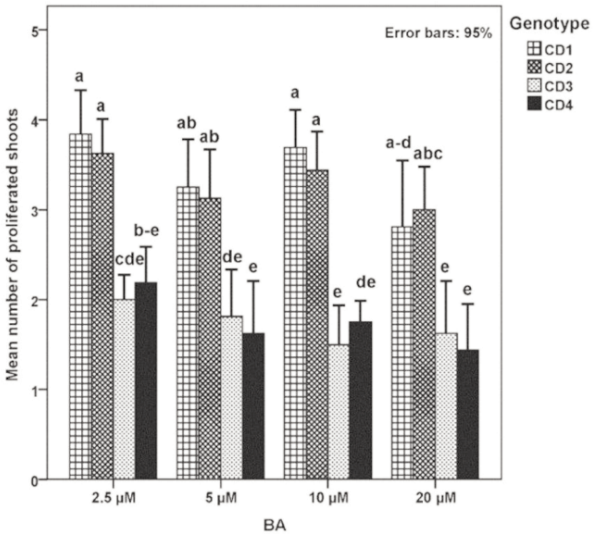

Fig. 4 - Effect of BA concentrations on shoot proliferation of four genotypes of *Cedrus deodara*. Bars with the same letters indicate no significant difference at Tukey test (P=0.05).

sues (Tang *et al.*, 2006; Humánez *et al.*, 2011). To our best knowledge, this is the first report of successful induction of axillary shoots on explants taken from adult *C. deodara.* There have been few reports on the effect of different kinds of basal media on the axillary shoot proliferation in cultures of explants from mature conifer trees (Andersone and Ievinsh, 2002; De Diego *et al.*, 2010; Renau-Morata *et al.*, 2005). In the present investigation, shoot proliferation rates were generally greater on basal WPM than on basal LP and MS media. Shoot buds cultured on MS medium presented the lowest organogenic response, and this was perhaps as a result of the comparatively high nitrate concentration as compared to the LP medium or WPM. Tuskan *et al.* (1990) showed that the extra nitrate had a negative effect on the organogenic response during micropropagation. Therefore, low nitrogen content of the medium is a major factor for promoting organogenesis in conifer species (Tang *et al.*, 2001; Schestibratov *et al.*, 2003). Explant type was an important factor affecting axillary bud proliferation in *in vitro* culture. Piola *et al.* (1998) demonstrated that the accumulation of ABA in needles seems to be the major cause of bud dormancy in micropropagation of *C. libani*. In this experiment, defoliated explants of *C. deodara* also showed better response on proliferation medium.

In *in vitro* bicentennial cedar micropropagation, it was found that the accomplishment of the protocol depends on genotype (Renau-Morata *et al.*, 2005). Among two cytokinin treatments examined, TDZ induced the highest number of proliferated shoots for all genotypes. After investigating the effect of three TDZ concentrations on shoot proliferation of four genotypes, the significant difference for their interaction was found. However, in our investigation, BA showed no significant genotypic differences for shoot proliferation. TDZ has gotten more attention in recent years due to its ability to assist *in vitro* regeneration of conifers (Mathur and Nadgauda, 1999; Sul and Korban, 2004; Renau-Morata *et al.*, 2005; Tang and Newton, 2005; Cortizo *et al.*, 2009; De Diego *et al.*, 2010; Humánez *et al.*, 2011). TDZ can decrease the enzyme activity related to oxidative stress during formation of adventitious shoots (Tang and Newton 2005). Recent reports on mature stone pine displayed the superiority of TDZ over other cytokinins in advancing axillary shoot proliferation (Cortizo *et al.*, 2009). It was shown that high concentrations of cytokinin in the medium, particularly BA led to the low regeneration response, which may be attributed to the toxic effects of high concentrations of cytokinins (Sarmast *et al.*, 2012). In this report, the presence of NAA with either TDZ or BA improved the incidence of shoot organogenesis in adult tissues of *C. deodara*. This has been also observed in other conifer species (Sul and Korban, 2004; Zhu *et al.*, 2010).

As this study shows, the type of explants and culture media has a very important role in success of culture establishment of *Cedrus deodara*. It was proven that defoliation of the explants has positive effect on shoot proliferation. The effects of genotype and growth regulators on proliferation of axillary

shoots have been studied and low concentration of TDZ combined with NAA has positive effects on the number of proliferated axillary shoots. Furthermore, two of the four genotypes showed better response to cytokinins.

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References

ANDERSONE U., IEVINSH G., 2002 - *Changes of morphogenic competence in mature* Pinus sylvestris *L. buds* in vitro. - Ann. Bot., 90: 293-298.

ANDERSONE U., IEVINSH G., 2005 - *In vitro regeneration of mature* Pinus sylvestris *buds stored at freezing temperatures.* - Biol. Plant., 49: 281-284.

BHATNAGAR S., SINGH M., KAPUR N., 1983 - *Preliminary investigations on organ differentiation in tissue cultures of* Cedrus deodara *and* Pinus roxburghii. - Indian J. Exp. Biol., 21: 524-526.

CHAMPION S.H., SETH S.K., KHATTAK G., 1965 - *Forest types of Pakistan.* - Pakistan Forest Institute, Peshawar, Pakistan, pp. 238.

CORTIZO M., DE DIEGO N., MONCALEÁN P., ORDÁS R.J., 2009 - *Micropropagation of adult stone pine (*Pinus pinea *L.).* - Trees, Struct. Funct., 23: 835-842.

DATTA M.M., MAJUMDER A., JHA S., 2006 - *Organogenesis and plant regeneration in* Taxus wallichiana *(Zucc.).* - Plant Cell Rep., 25: 11-18.

DE DIEGO N., MONTALBÁN I., MONCALEÁN P., 2010 - In vitro *regeneration of adult* Pinus sylvestris *L. trees.* - South Afr. J. Bot., 76: 158-162.

DUMAS E., MONTEUUIS O., 1995 - In vitro *rooting of micropropagated shoots from juvenile and mature* Pinus pinaster *explants: influence of activated charcoal.* - Plant Cell, Tiss. Org. Cult., 40: 231-235.

GUO B., ABBASI B.H., ZEB A., XU L.L., WEI Y.H.,2011 - *Thidiazuron: a multi-dimensional plant growth regulator.* - Afr. J. Biotechnol., 10: 8984-9000.

GUPTA P.K., DURZAN D.J., 1985 - *Shoot multiplication from mature trees of Douglas-fir (*Pseudotsuga menziesii*) and sugar pine (*Pinus lambertiana*).* - Plant Cell Rep., 4: 177-179.

HUMÁNEZ A., BLASCO M., BRISA C., SEGURA J., ARRILLAGA I., 2011 - *Thidiazuron enhances axillary and adventitious shoot proliferation in juvenile explants of Mediterranean provenances of maritime pine* Pinus pinaster. - In Vitro Cel. Dev. Biol. Plant, 47: 569-577.

LIN Y., WAGNER M.R., HEIDMANN L., 1991 - In vitro *formation of axillary buds by immature shoots of Ponderosa pine.* - Plant Cell, Tiss. Org. Cult., 26: 161-166.

LLOYD G., McCOWN B., 1980 - *Commercially-feasible micropropagation of mountain laurel*, Kalmia latifolia, *by use of shoot-tip culture.* - Combined Proceedings IPPS, 30: 421-427.

MALABADI R.B., VAN STADEN J., 2005 - *Somatic embryogenesis from vegetative shoot apices of mature trees of* Pinus patula. - Tree Physiol., 25: 11-16.

MATHUR G., NADGAUDA R., 1999 - In vitro *plantlet regeneration from mature zygotic embryos of* Pinus wallichiana *AB Jacks.* - Plant Cell Rep., 19: 74-80.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bio assays with tobacco tissue cultures.* - Physiol. Plant., 15: 473-497.

PARASHARAMI V.A., POONAWALA I.S., NADGAUDA R.S., 2003 - *Bud break and plantlet regeneration* in vitro *from mature trees of* Pinus roxburghii *Sarg*. - Curr. Sci., 84: 203-208.

PIERIK R.L.M., 1987 - In vitro *culture of higher plants.* - Martinus Nijhoff, Dordrecht, The Netherlands.

PIOLA F., LABEL P., VERGNE P., VON ADERKAS P., ROHR R., 1998 - *Effects of endogenous ABA levels and temperature on cedar (*Cedrus libani *Loudon) bud dormancy* in vitro*.* - Plant Cell Rep., 18: 279-283.

PIOLA F., ROHR R., 1996 - *A method to overcome seed and axillary bud dormancy to improve* Cedrus libani *micropropagation.* - Plant Tiss. Cult. Biotechnol., 2: 199-201.

PIOLA F., ROHR R., HEIZMANN P., 1999 - *Rapid detection of genetic variation within and among in vitro propagated cedar(*Cedrus libani Loudon*) clones.* - Plant Sci., 141: 159-163.

QUOIRIN M., LEPOIVRE P., 1977 - *Etude de milieux adaptes aux cultures* in vitro *de Prunus.* - Acta Horticulturae, 78: 437-442.

RENAU-MORATA B., OLLERO J., ARRILLAGA I., SEGURA J., 2005 - *Factors influencing axillary shoot proliferation and adventitious budding in cedar.* - Tree Physiol., 25: 477-486.

SARMAST M.K., SALEHI H., RAMEZANI A., ABOLIMOGHADAM A.A., NIAZI A., KHOSH-KHUI M., 2012 - *RAPD fingerprint to appraise the genetic fidelity of* in vitro *propagated* Araucaria excelsa R. Br. var. *glauca plantlets.* - Mol. Biotechnol., 50: 181-188.

SCHESTIBRATOV K.A., MIKHAILOV R.V., DOLGOV S.V., 2003 - *Plantlet regeneration from subculturable nodular callus of* Pinus radiate. - Plant Cell, Tiss. Org. Cult., 72: 139-146.

SUL I.W., KORBAN S.S., 1994 - *Effect of different cytokinins on axillary shoot proliferation and elongation of several genotypes of* Sequoia sempervirens. - In Vitro Cell. Dev. Biol. Plant, 30: 131-135.

SUL I.W., KORBAN S.S., 2004 - *Effects of salt formulations, carbon sources, cytokinins, and auxin on shoot organogenesis from cotyledons of* Pinus pinea *L*. - Plant Growth Regul., 43: 197-205.

TAMTA S., PALNI L., 2004 - *Studies on* in vitro *propagation*

*of Himalayan cedar (*Cedrus deodara*) using zygotic embryos and stem segments.* - Indian J. Biotechnol., 3: 209-215.

TANG W., GUO Z., OUYANG F., 2001 - *Plant regeneration from embryogenic cultures initiated from mature loblolly pine zygotic embryos.* - In Vitro Cell. Dev. Biol. Plant, 37: 558-563.

TANG W., NEWTON R.J., 2005 - *Peroxidase and catalase activities are involved in direct adventitious shoot formation induced by thidiazuron in eastern white pine (*Pinus strobus L.*) zygotic embryos.* - Plant Physiol. Biochem., 43: 760-769.

TANG W., NEWTON R.J., CHARLES T.M., 2006 - *Plant regeneration through multiple adventitious shoot differentiation from callus cultures of slash pine (*Pinus elliottii*).* - J. Plant Physiol., 163: 98-101.

TEWARI D.N., 1994 - *A monograph on deodar* (Cedrus deodara *(Roxb.) G. Don*). - International Book Distributors, Dehra Dun, India, pp. 213.

TUSKAN G., SARGENT W., RENSEMA T., WALLA J., 1990 - *Influence of plant growth regulators, basal media and carbohydrate levels on the* in vitro *development of* Pinus ponderosa *(Dougl. ex Law.) cotyledon explants.* - Plant Cell, Tiss. Org. Cult., 20: 47-52.

VASIL I.K., VASIL V., 1980 - *Clonal propagation*. - Int. Rev. Cytol. Suppl., 11A: 146-173.

ZHU L.H., WU X.Q., QU H.Y., JI J., YE J.R., 2010 - *Micropropagation of* Pinus massoniana *and mycorrhiza formation* in vitro. - Plant Cell, Tiss. Org. Cult., 102: 121-128.